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Research Article

Following in the footsteps of invasion: comparisons of founder and invasive genotypes of two independent invasions reveal site-specific demographic processes and no influence by landscape attributes on dispersal

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Abstract

To understand the success of invasive alien species, it is necessary to evaluate the site-specific eco-evolutionary challenges they face in their new environments. We explored whether the rearrangement of genetic diversity is linked to the invasiveness of *Prosopis juliflora* by (i) comparing different stages of invasion (founding vs invasive populations) in two invaded areas (Afar Region, Ethiopia and Baringo County, Kenya) to evaluate whether different stages are dominated by different genetic attributes (e.g., characteristic genotypes or levels of genetic diversity) and by (ii) evaluating if landscape features affected dispersal between invasive populations in the two invaded areas. We hypothesised that different invasion stages would have unique genetic characteristics due to either site-specific demographic and/or dispersal dynamics. We also compared the genetic characteristics at an 'invasive–non-invasive congener' level by studying the non-invasive *P. pallida*, introduced to Baringo County, and assessed whether it hybridises with *P. juliflora*. In the Afar Region, the establishment and spread of *P. juliflora* were characterised by extensive gene flow that homogenised genetic diversity across all populations. In contrast, in Baringo County, invasive populations had lower genetic diversity than founders, and genetic differentiation was lower between invasive populations than between invasive and founder populations. In both invaded areas, we found no evidence that dispersal was hampered by geographic distance, bioclimatic conditions, or distance to roads, rivers and villages, at least at the spatial scales of our study; indicating frequent long-distance dispersal. Allelic richness was higher in *P. juliflora* than *P. pallida* founders and hybrids were mainly planted trees probably resulting from the sympatric cultivation of the two species following their introduction. Thus, management actions on *Prosopis* invasion in eastern Africa should consider site-specific dynamics occurring during the invasion.

Key words: demographic stochasticity, dispersal, invasive spread, microsatellites, *Prosopis*, woody invasive species

Introduction

The genetic determinants of the success of invasive alien species have been long recognised (Vignieri 2005; Ward et al. 2008; Zenni et al. 2017; Le Roux 2021). Genetic studies on naturalised and invasive populations have also contributed to our understanding of the drivers of invasion success, including the factors that underlie establishment and spread (Baker and Stebbins 1965; Le Roux, 2021). This knowledge, in turn, has provided important information for predicting the eco-evolutionary dynamics of invasive populations to assist their management (Funk et al. 2020; Morisette et al. 2021; Byrne et al. 2022). For example, genetic analyses can be used to infer patterns of past spread (Cushman 2015), modes of dispersal (long- vs short-distance; Sexton et al. 2014; Smith et al. 2020), or to understand the stochastic demographic events (i.e., genetic drift) or evolutionary processes (i.e., selection) that have shaped the genetic diversity present in invasive populations (Dlugosch and Parker 2008; van Boheemen et al. 2018; Le Roux 2021).

Demographic processes directly affect patterns of genetic variation (Loog 2021), for example, by determining the strength by which genetic drift rearranges diversity within and among populations (Caballero 1994). In the case of alien species, introductions can be characterised by founder events and subsequent genetic bottlenecks, and thus reduced genetic variation (Henry et al. 2009; Shirk et al. 2014; but see Marrs et al. 2008; Estoup et al. 2016; Smith et al. 2020). Higher levels of invasiveness are normally associated with higher levels of genetic diversity, because how alien species respond to novel selection regimes depends, partly, on the amount of standing genetic diversity present in introduced populations (Ward et al. 2008). Frequently, invasive spread is preceded by a lag phase of relatively small population sizes. These periods of minimal spread are thought to, in part, reflect the time needed for introduced populations to replenish genetic diversity and overcome demographic processes that negatively affect population growth, such as Allee effects (Zenger et al. 2003; Bousset et al. 2004). Inter- and intraspecific hybridisation (the last hereafter referred to as genetic admixture) may also help replenish genetic variation in founding populations and to create novel and heritable genetic variation that can fuel invasiveness via rapid evolution (Ellstrand and Schierenbeck 2000), while polyploid alien species are often more successful invaders than diploid ones (te Beest et al. 2012).

Many alien species undergo rapid evolution in their new ranges to become invasive (Blossey and Nötzold 1995; Maron et al. 2007; Ochocki and Miller 2017; van Boheemen et al. 2018; Castillo et al. 2021b; Le Roux 2021). For example, climate conditions may differ substantially between a species' historical and new ranges, imposing strong selection pressure. On the other hand, species may experience relaxed selection when they are liberated from their specialist predators or parasites in the new range, i.e., historical selection pressures are either eliminated or dramatically reduced (Blossey and Nötzold 1995). It is conceivable that different stages of invasion, i.e., founder vs invasive populations, will comprise different genotypes, which may be indicative of contemporary genetic change during invasion. Such evolutionary responses are typically inferred by comparing 'ancestral' (i.e., native range) and 'descendant' (i.e., invasive) populations (Keller and Taylor 2008). However, the actual ancestors are likely not included in such studies.

Dispersal is a central factor in relating microevolutionary processes to landscape variables, because the movement and successful establishment of propagules (i.e., survival and reproduction) determines the structuring of genetic variation within and

between invasive populations (Pflüger and Balkenhol 2014). This allows for indirect inferences of how landscape attributes influence the dispersal dynamics between populations, by relating the spatial distribution of genetic variation to landscape variables. To this end, landscape resistance models (i.e., spatial hypotheses on how landscape features influence gene flow) are normally used to examine the effects of landscape variables and geographic distance on the distribution of allele frequencies between populations across landscapes (Broquet and Petit 2009; Pflüger and Balkenhol 2014; Balkenhol et al. 2015). These genetic studies have been helpful in elucidating the underlying processes and patterns associated with dispersal (Lenormand 2002; Bridle et al. 2010; Berthouly-Salazar et al. 2012; Cahill and Levinton 2016), but there is a need for broader approaches that integrate information on the spatial genetic structure and genetic differentiation of invasive populations (as a proxy for dispersal) with their probability to establish. For example, knowing which landscape variables affect dispersal patterns between populations (i.e., facilitating or restricting the gene flow between populations) is of limited management value if considered in isolation, especially in heterogeneous habitats; as invasive spread ultimately depends on the availability of suitable habitat(s) (Le Roux et al. 2010; Meier et al. 2014). Similarly, suitable habitats alone would not guarantee the establishment of a particular species if limitations in dispersal hinder propagules from reaching these habitats. Under these circumstances, linking estimates of dispersal distances to suitable habitats in the introduced area offers more realistic insights into the rapid range expansion of species and the microevolutionary processes that accompany invasion.

Prosopis invasions in eastern Africa offer excellent opportunities to examine some of the genetic attributes of invasion success discussed above. The founder trees of two species, the invasive *Prosopis juliflora* (Sw.) DC. and non-invasive *Prosopis pallida* (Willd.), are still present in the original plantations today (Choge et al. 2002; Mbaabu et al. 2019; Shiferaw et al. 2019; Castillo et al. 2021a,b). This makes it possible to examine (1) the genotypes that acted as sources of widespread invasions in the region, providing a unique opportunity to use genetic approaches to evaluate the dynamics underlying invasion success and (2) the genetic attributes of invasive and non-invasive congeners found in the same environment. To our knowledge, no such studies exist. Notably, in Baringo County, Kenya, *P. juliflora* and *P. pallida* were introduced and share the same residence time and propagule pressure under similar local environmental conditions (Choge et al. 2002). Importantly, differences in functional traits and plastic responses between planted and invasive conspecific individuals due to post-introduction evolution, and between planted individuals of *P. juliflora* and *P. pallida*, may have enabled the transition from successful naturalisation to invasive spread in *Prosopis* (Castillo et al. 2021b).

In both Ethiopia and Kenya, the most relevant environmental variables that explain the current and future distributions of *Prosopis* include elevation, rivers, roads and bioclimatic conditions related to temperature and precipitation (Eckert et al. 2020; Shiferaw et al. 2019; Mbaabu 2023). Similarly, worldwide, the movement of *Prosopis* propagules seems to be affected by linear landscape variables (e.g., rivers and roads; Pasiecznik et al. 2001; Shiferaw et al. 2004). This provides the opportunity to elucidate the determinants of dispersal in these trees by using resistance models with environmental variables that are ecologically relevant for the occurrence and invasive spread of *Prosopis* species.

Here, we explored whether the introduction and spread of *Prosopis* in eastern Africa have been accompanied by changes in genetic diversity. We hypothesised that

different invasion stages (founder vs spreading populations) will be dominated by different genetic characteristics due to either site-specific demographic processes and/or dispersal dynamics (Fig. 1). To test this hypothesis, we addressed the following research questions (1) whether founder and invasive populations of *P. juliflora*, in two invaded areas, are dominated by different genotypes (i.e., different alleles and allele frequencies), indicative of contemporary genetic change during rapid range expansion; (2) whether dispersal between invasive populations, in the two invaded areas, is similarly affected by environmental variables. We did this by testing whether variables that are known to influence the occurrence and spread of *Prosopis* in eastern Africa are related to the spatial distribution of genetic variation between *Prosopis* populations (and thus dispersal). (3) Lastly, we evaluated whether the two *Prosopis* species, differing in ploidal levels and invasiveness, differ in their genetic characteristics and assessed the existence of hybrids between them. We hypothesised that polyploid species will exhibit greater genetic variation as a result of genomic duplication.

Methods

Study sites and species

This study was carried out in two areas in the Great Rift Valley of eastern Africa, the Afar Region in Ethiopia, and Baringo County in Kenya (Fig. 2). The Afar Re-

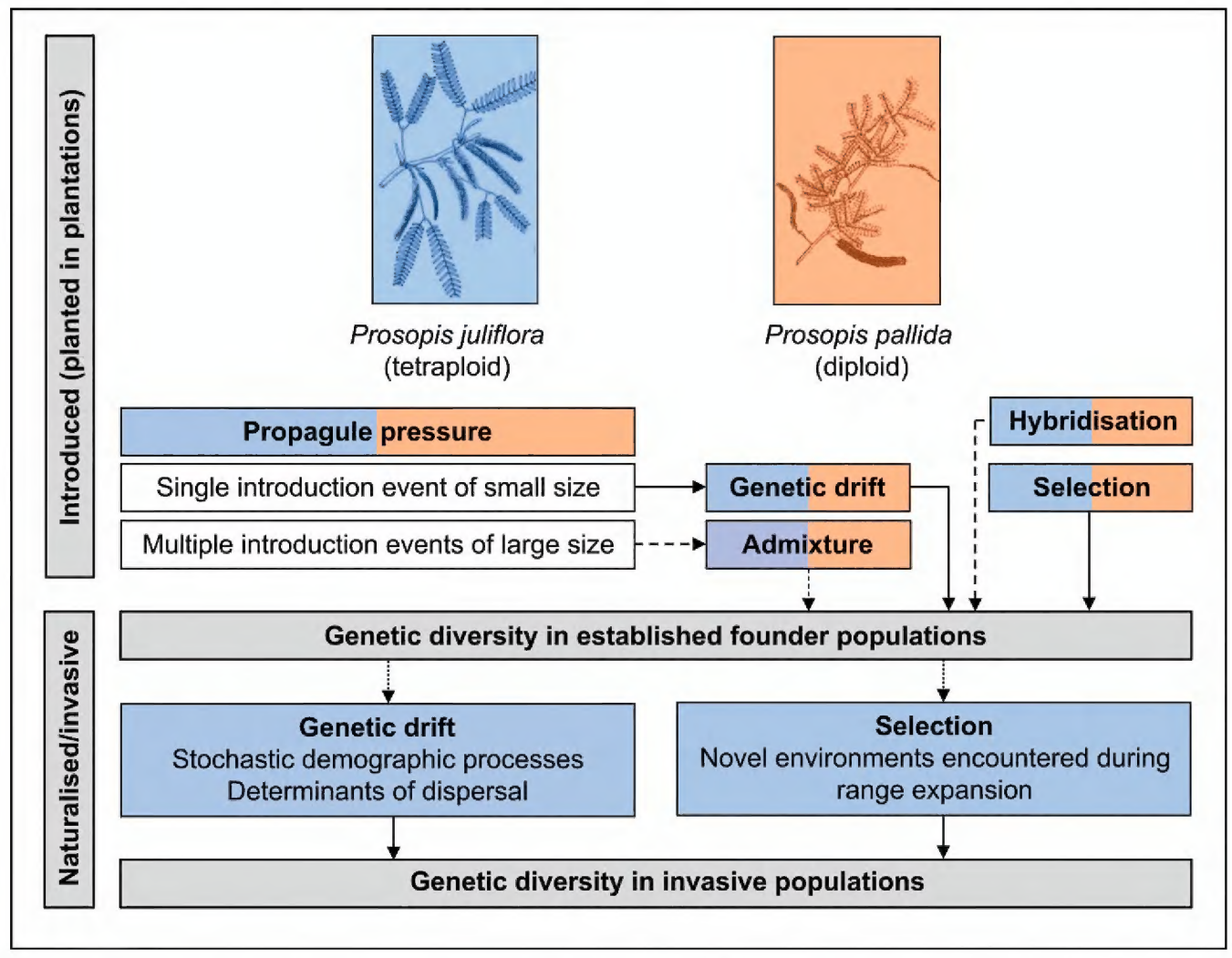

Figure 1. Summary of the eco-evolutionary dynamics of *Prosopis* invasions related to this study. Our research questions relate to the invasion dynamics of introduced invasive (blue) and non-invasive (orange) *Prosopis* species in eastern Africa. We evaluated (directly or indirectly) whether various factors (in bold in horizontal boxes), associated with different stages of invasion, have shaped the genetic characteristics of populations in the invaded areas of Afar Region, Ethiopia and Baringo County, Kenya. These factors can either decrease (solid arrows) or increase (dashed arrows) genetic diversity found in founder and invasive populations. Note that factors associated with invasive populations could only be assessed for *P. juliflora*. Figure modified from Keller and Tailor (2008).

gion is one of the main pastoral regions in eastern Africa, located in north-eastern Ethiopia. The region comprises hilly escarpments of up to 1500 m a.s.l., and the valley of the Awash River, which flows through the region to the Ethiopia-Djibouti border with altitudes between -155 to 100 m.a.s.l. (Gasse and Street 1978). The mean annual temperature is 31 °C and summer temperatures can rise up to 41 °C. Mean annual precipitation is 560 mm, with July and August being the wettest months (WAS 2000). The area is characterised by a fragmented landscape with arid savanna vegetation (MOA 1997).

Baringo County is located in western Kenya just north of the equator. Land in the area is principally used for livestock farming and cropping. Elevation ranges between 700 and 3000 m.a.s.l. in the area (Keitany et al. 2013) and its climate varies from humid in the highlands to arid in the lowlands. Mean annual temperature ranges from 10 °C to 35 °C and mean annual precipitation ranges from 500 mm to 1000 mm with two peaks in rainfall in April and November (Kipkorir 2002).

Prosopis was first introduced into the Afar Region in the early 1980s at Amibara and Gewane districts (Admasu 2008; Kebede and Coppock 2015) with additional introductions between the mid 1980s and 1990s as shade trees in villages and to control erosion (Kebede and Coppock 2015). In Kenya, *P. juliflora* and *P. pallida* trees sourced from Brazil and Hawaii were first introduced in 1973 to Mombasa to rehabilitate quarries (Johansson 1990), with later introductions during the 1970s

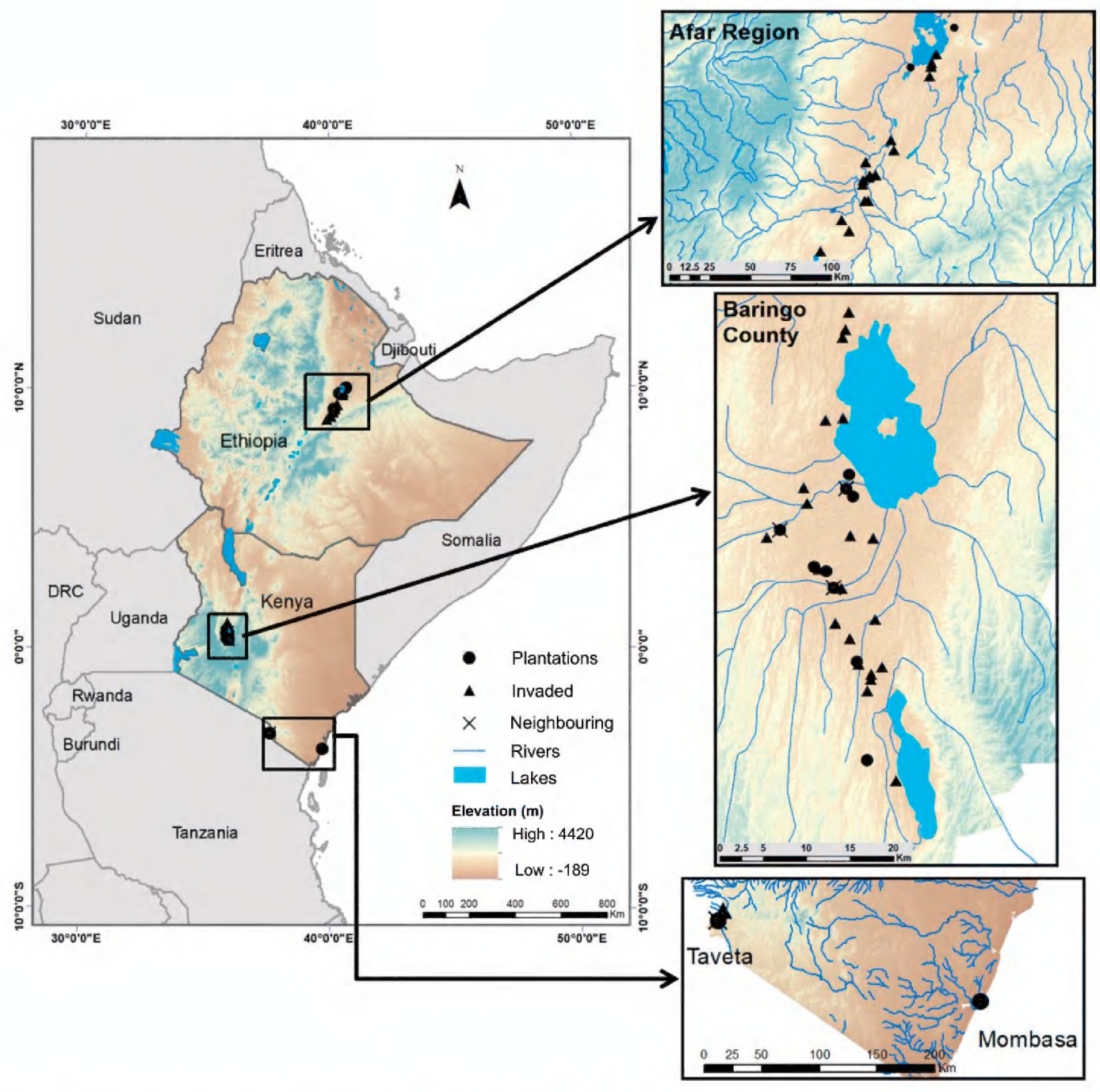

Figure 2. Location of the study areas in Ethiopia (Afar Region) and Kenya (Baringo County, Mombasa and Taveta). Invaded sites are > 100 m, and neighbouring sites < 100 m from plantations (i.e., introduced founder individuals), respectively (see Suppl. material 1 for further details).

and 1980s to many parts of Kenya, including Baringo County, Tana River and Taveta (Johansson 1990; Otsamo and Maua 1993; Choge et al. 2002). During this time, demonstration plantations of *Prosopis* species were established using seeds sourced from a few individuals of each species (Choge et al. 2002). *Prosopis* has since become invasive in both countries causing substantial social-economic conflicts (Swallow and Mwangi 2009; Kebede and Coppock 2015; Bekele et al. 2018) and negatively impacting biodiversity and ecosystem functioning (Linders et al. 2019).

Pasiecznik et al. (2001) considered *Prosopis juliflora* and *P. pallida* to be a species complex because of their similar morphology and environmental tolerances. Recently, a phylogenetic study reclassified *Prosopis* species into four genera, with *P. juliflora* and *P. pallida* now assigned to the genus *Neltuma* as *Neltuma juliflora* (Sw.) Raf., Sylva Tellur and *N. pallida* (Humb. & Bonpl. ex Willd.) C.E. Hughes & G.P., respectively (Hughes et al. 2022). *Prosopis* trees are primarily insect-pollinated and are generally assumed to be self-incompatible, but limited self-compatibility has been observed for *P. juliflora* (Pasiecznik et al. 2001). Seeds produced during the wet season are mainly dispersed by flood water while in the dry season, pods are consumed and seeds are largely dispersed by ungulates (Pasiecznik et al. 2001). Human-assisted dispersal has been associated with the presence of the species in settlements and roads (Pasiecznik et al. 2001; Shiferaw et al. 2004; Muturi 2012).

Sample collection

In the Afar Region, *Prosopis* leaf material was collected from 202 individuals randomly chosen from 22 sites, including five plantations containing the founder individuals introduced into the area and 17 invaded sites (Suppl. material 1; Fig. 2a). Target trees from invaded sites were at least 100 m from plantations to meet the definition proposed by Richardson et al. (2000) for invasive spread that "reproductive offspring" should be more than 100 m away from adults. We randomly sampled between eight and 20 trees from each plantation and between three and 15 individuals from each invaded site. All individuals were morphologically identified as *P. juliflora* (see Results section).

In Baringo County, we randomly sampled 504 individuals from 44 sites (Suppl. material 1; Fig. 2b). Besides sites representing founder and more distant invasive populations, we also sampled "reproductive offspring" at less than 100 m from founder individuals (hereafter sites neighbouring plantations) as they may include hybrids between *P. juliflora* and *P. pallida* (Castillo et al. 2021a). Overall, we sampled individuals from nine plantations, three sites from neighbouring plantations (six of them did not have "reproductive offspring"), and 32 invaded sites.

In addition, for Kenya, we included samples from one plantation each at the outskirts of Mombasa City and Taveta Town, one site neighbouring the Taveta plantation, and one invaded site in Taveta. The Mombasa plantation is located on the south-eastern coast of Kenya, whereas the Taveta plantation is situated in south-western Kenya, at the border with Tanzania (Suppl. material 1; Fig. 3c). From Baringo County, Mombasa and Taveta plantations individuals were randomly chosen. Between one and 44 founder trees of *P. pallida* and *P. juliflora* were sampled (four plantations in Baringo County only had one founder *P. pallida* tree left). In Baringo County and Taveta, at sites of neighbouring plantations and invaded sites, between one and 25 trees were sampled. The morphotype of sampled individuals was recorded as either *P. juliflora*, *P. pallida* or putative hybrid. Identifi-

cation of these morphotypes was based on broad morphological differences in stem structure, leaf morphology, pod shape, and the presence or absence of thorns (Burkart 1976; Pasiecznik et al. 2001; Castillo et al. 2021a). Individuals with intermediate characteristics of the two parental species were classified as putative hybrids. Further details on the morphological classification of individuals are provided in Castillo et al. (2021a). Sampling in the two study areas was conducted between September 2016 and March 2017.

We collected samples across the invaded area in both study sites, covering the entire range of environmental variables known to influence the occurrence and spread of *Prosopis* (e.g., temperature and precipitation, altitude) as well as at different distances from rivers, roads and villages (Shiferaw et al. 2019; Mbaabu 2023). The geographic location of each collection site was recorded using a handheld GPS receiver (Suppl. material 1). For simplicity, throughout this manuscript, the term population will be used to refer to the group of individuals sampled at a single collection site.

DNA extraction and microsatellite genotyping

Leaf material was air-dried and stored on silica gel until DNA extraction. Genomic DNA was extracted from dried leaf tissue using the cetyltrimethylammonium bromide protocol (Doyle and Doyle 1990). All DNA extractions were diluted to a final concentration of 50 ng/μL and stored at -20 °C until further analysis. Individuals from eight sites in Kenya (KEN3, KEN6, KEN35, KEN37, KEN41, KEN42, KEN43 and KEN44; n = 14; Suppl. material 2) were not genotyped and samples were only included in flow cytometry analyses (see below).

Individuals were genotyped at seven microsatellite markers: Gl12, I-P06639, Prb4, Prsc7, Prsc9, S-P1DKSFA and S-P1EPIIV2. These markers were selected based on successful PCR amplification in *P. juliflora* and *P. pallida* (see Castillo et al. 2021a). The amplification of these seven markers was performed by using one multiplex PCR reaction (see Castillo et al. 2021a for further details). Multiplex PCR reaction contained 1.5 μL of primer mix (2 μM), 7.5 μL of KAPA2G Fast Multiplex Mix (Kapa Biosystem, Cape Town, South Africa), 4.5 μL purified H_2O and 1.5 μL of DNA making a total of 15 μL of volume solution (see Castillo et al. 2021a for information on the volume of each microsatellite primer and PCR cycle). Each 96-well PCR plate contained 89 samples plus five randomly selected technical replicate samples and two negative controls (H_2O). Amplified products were submitted for gel capillary electrophoretic separation at the Central Analytical Facility, Stellenbosch University, South Africa. GeneMarker software v2.6.4 (SoftGenetics LLC, Pennsylvania, United States) was used for automated genotype scoring, which was then manually checked. Out of 730 samples, 19 samples that failed to amplify at more than five loci were excluded from the analysis.

Polyploidy, hybridisation and population genetic structure

Because of differences in ploidy between *P. juliflora* (2n = *4x*) and *P. pallida* (2n = *2x*), we estimated the genome sizes of *Prosopis* individuals using flow cytometry analysis on a structured random subset of our sampled individuals (i.e., to include individuals from each invaded area and invasion stage). For this analysis, we included 10 individuals from the Afar Region (six founder trees and four in-

vaders), representing both plantations (n = 4) and invaded sites (n = 4), and 53 individuals from Baringo County (25 founder trees and 28 invaders), representing plantations (n = 6) and invaded sites (n = 13). Samples from Baringo County represented morphotypes of both *P. pallida* (n = 12) and *P. juliflora* (n = 41; Suppl. material 2). During the field survey, no putative hybrids were detected based on morphology only (see Results section). For flow cytometry, the method of Temsch et al. (2010) was followed, using 1 cm^3 of mature leaf sample per individual and *Solanum pseudocapsicum* L. as the internal standard. A Pearson's Chi-squared test was performed to evaluate the association between the cytotype of individuals and their frequency in invaded sites in Baringo County only (see Results section).

Population genetic structure and the presence of hybrids were estimated using the STRUCTURE software v2.3.4 (Pritchard et al. 2000). STRUCTURE uses Bayesian Monte–Carlo Markov chain sampling to identify the optimal number of genetic clusters for a given dataset by reducing departures from Hardy–Weinberg and linkage equilibrium expectations within genetic clusters. For a first "overall" STRUCTURE analysis, two genetic clusters (*K*), corresponding to *P. juliflora* and *P. pallida* were tested and 10 independent models for $K = 2$ were run. Each model consisted of 500,000 generations of which the first 100,000 were discarded as burn-in. Due to the probable presence of hybrids, an admixture model with correlated allele frequencies was specified. For datasets including individuals with different ploidy levels, STRUCTURE requires an overall ploidy to be specified. In this case, an overall ploidy of 4x was used. Following (Pritchard et al. 2010) for analyses including polyploid individuals, the option RECESSIVEALLELES was set to one to account for allele copy ambiguity and for diploid and triploid individuals, and a missing data symbol was added to complete the ploidy level. This indicates that the individual is diploid or triploid at all the loci. STRUCTURE provides assignment values for each individual to the different genetic clusters tested, calculated as the proportion (q_{ik}) of each individual genotype assigned to each of the optimal number of genetic clusters. These assignment values were used to determine the presence of hybrids, with individuals having similar membership to both genetic clusters being classified as F1 hybrids. We expected all putative F1 hybrids to be sterile due to their triploid genomes, and therefore the assignment values (q_{ik}) to each genetic cluster to be close to 0.5.

To infer site-specific demographic processes, we evaluated the number of genetic clusters present in the invaded area of both study sites. Thus, a second STRUCTURE analysis was run that included only *P. juliflora* individuals. For this "*P. juliflora*–only" analysis (630 individuals; see Results section), triploid individuals identified by flow cytometry analyses, hybrids identified by the first STRUCTURE analysis (see above and Results section), and *Prosopis pallida* trees (identified based on morphology, flow cytometry and results from the "overall" STRUCTURE analysis; see Results section) were excluded. We ran models with similar parameters as described above for the "overall" analysis but specifying *K* values ranging between one to 20. The optimal *K* value was estimated according to the method of Evanno et al. (2005), using the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). CLUMPAK software (Kopelman et al. 2015) was used to graphically display the results.

Population genetic structure was further assessed in two separate principal component analyses (PCAs), one with the "overall" and one with the "*P. juliflora*-only" datasets (see above) using the R packages PolySat (Clark and Jasieniuk

2011) and FactoMineR v2.6 (Lê et al. 2008). The PolySat R package allows for the inclusion of microsatellite data of any ploidy level, including populations with mixed ploidy levels. For this, a matrix of pairwise distances between individuals was generated using Bruvo distances (Bruvo et al. 2004). This method was preferred because it incorporates distances between microsatellite alleles without information on allele copy number (Bruvo et al. 2004). For the PCA on the "overall" dataset, we incorporated species morphotype (i.e., *P. juliflora* or *P. pallida* morphotypes), invasion stages (i.e., plantations vs invaded sites) and country as supplementary variables to examine their association with the principal components. In the PCA using the "*P. juliflora*–only" dataset, we added invasion stages and country as supplementary variables.

Genetic insights from different invasion stages

To infer site-specific demographic processes, we examined whether genetic characteristics differed between different invasion stages in both study areas by calculating various genetic diversity statistics: allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E; corrected for sample size, Nei 1978), and inbreeding coefficients (F_{IS}); for each population using SPAGEDI software v1.5 (Hardy and Vekemans 2002). These metrics were compared across invasion stages for *P. juliflora* individuals (using the same dataset as for the "*P. juliflora*-only" analysis) in Baringo County (i.e., plantations, sites neighbouring plantations and invaded sites) and the Afar Region (i.e., plantations and invaded sites), and for *P. pallida* individuals from Baringo County (i.e., plantations and sites neighbouring plantations). These analyses were performed using a nonparametric bootstrap t-test due to the non-normal distribution of data and the small size of some samples (Dwivedi et al. 2017).

Site-specific demographic processes were also inferred by examining the genetic differentiation between different invasion stages in both study areas. For this, we calculated pairwise fixation indices (F_{ST}) between *P. juliflora* populations in Baringo County and the Afar Region separately using the PolySat R package. We used the 'deSilvaFreq' function to estimate allele frequencies. This method considers "allelic phenotypes" instead of genotypes to estimate allele frequencies, assuming random mating and either disomic or polysomic inheritance without double reduction (De Silva et al. 2005). For partial heterozygous genotypes, this approach assumes that all alleles have an equal chance of having more than one copy. It also enables the inclusion of selfing rates in the analysis, which we specified as 0.04 (Sareen and Yadav 1987). We use the same dataset as for the "*P. juliflora*-only" analysis and sites. Sites with only one sampled individual were excluded from these analyses. We then tested for pairwise genetic differences between all pairs of populations from the same and different invasion stages in Baringo County (plantations, neighbouring and invaded sites) and the Afar Region (plantations and invaded sites) separately by using Kruskal-Wallis tests and Dunn tests for post hoc multiple comparisons. In addition, to evaluate how genetic variance is partitioned among the invasion stages in each study area, an analysis of molecular variance (AMOVA) was performed using pairwise distances between individuals generated with Bruvo distances (Bruvo et al. 2004). For this, we tested the genetic variance in Baringo County and the Afar Region separately, with the pegas R package v0.11 (Paradis 2010) at three levels: among invasion stages (populations nested within invasion stages), among populations and within populations.

Effect of environmental variables on dispersal at invasive population level

To evaluate the effect of landscape and climatic variables on dispersal, we estimated the genetic differentiation between populations as pairwise fixation indices (F_{ST}) using the 'deSilvaFreq' function implemented in the PolySat R package. This was done for *P. juliflora* individuals from Baringo County and the Afar Region separately. Since individuals from plantations have been planted in each study site and are the source of invasive *P. juliflora*, pairwise F_{ST} values were calculated considering only individuals from invaded sites in the Afar region, and individuals from invaded and sites neighbouring plantations for Baringo County. Sites with only one sampled individual were excluded. In the case of the Afar Region, the locus S-P1EPIIV2 was monomorphic in invasive individuals, so the analyses were conducted using the remaining six microsatellite markers only.

We used landscape resistance modelling to infer how various environmental variables and geographic distance influence gene flow (i.e., pairwise F_{ST} values), and thus, indirectly dispersal, between invasive *Prosopis* populations in both study areas. Geographic distances between populations were calculated from GPS coordinates with the 'pointDistance' function in the raster R package v3.6-3 (Hijmans 2022). Environmental variables that are known to influence the current presence and spread of *Prosopis* in each study area could likely affect the dispersal between invasive populations. Therefore, we gathered these variables following Shiferaw et al. (2019) and Mbaabu (2023) for the Afar Region and Baringo County (Suppl. material 3). For both study areas, we considered that distance to rivers, roads, villages and lower annual precipitation affect dispersal negatively. We hypothesised that dispersal is lower (i.e., higher pairwise F_{ST} values) at a higher altitude in the Afar Region but is favoured in Baringo County (altitude is positively correlated with mean annual precipitation; results not shown). For Baringo County, following Mbaabu (2023), we also hypothesised that dispersal is favoured (i.e., lower pairwise F_{ST} values) at higher precipitation of the wettest month; and at lower mean temperature of the driest quarter, maximum temperature of the warmest month and distance to markets. The spatial resolution of the landscape layers was the same for both study areas (i.e., 60 m). We applied a monomolecular transformation to cell values of the linear landscape variables: distance to markets, rivers, roads and villages; using the ResistanceGA R package (Peterman 2018). The monomolecular transformation follows an exponential function $y = r\ (1 - exp^{-bx})$; where r is the resistance surface, x is the shape and b is the magnitude parameter (see Peterman 2018 for details). By using this transformation, we hypothesised that resistance values increase exponentially (i.e., assuming a shape of one and a maximum value of resistance of 100) with higher values of the variable (e.g., markets and villages are highly permeable to dispersal). For the rest of the environmental variables of both countries (i.e., elevation, or related to temperature and precipitation), we applied an inverse-reverse monomolecular transformation (with the same shape and maximum value of resistance as above) so that we have a monotonical increase or decrease of resistance values with higher or lower values of the environmental variable (e.g., resistance values decrease monotonically with higher values of mean annual precipitation).

Least-cost path approaches were used for modelling dispersal on the resistance surfaces generated above. This approach correlates genetic distances (i.e., pairwise F_{ST} values) with ecological distances along the shortest, single suitable path between sites considering each resistance surface (i.e., path with the lower resistance

values; Vignieri 2005). The 'Costdistance' function within the gdistance R package v1.2-2 (van Etten 2017) was used to calculate the ecological distance between sites. Finally, to assess the effect of environmental variables on the dispersal of *Prosopis* in both invaded areas, Mantel tests were carried out for each country to assess the correlation between genetic distances (linearised pairwise F_{ST} values = F_{ST}/1 - F_{ST}) and geographic and ecological distances respectively using the vegan R package v2.6-4 (Oksanen et al. 2022). Geographic and ecological distances were ln-transformed to conform to Mantel test assumptions of linearity in the relationship with the genetic distances (Legendre et al. 2015). Analyses with the R statistical language were done on R software v3.4.3.

Results

Polyploidy, hybridisation and population genetic structure

Morphological identification of trees suggested that only *P. juliflora* individuals were present in invaded sites in both Kenya and Ethiopia. *Prosopis pallida* was only recorded in plantations and in one neighbouring site in Baringo County (Suppl. material 1). Flow cytometry analysis showed all individuals in the Afar Region that were morphologically identified as *P. juliflora* were tetraploid (i.e., relative to genome size of standard, hereafter rel. gen. size, 1.81 ± 0.03; n = 3) and triploid (rel. gen. size 1.38 ± 0.04; n = 4), the latter found in similar number in both plantations and invaded sites. Diploid individuals (rel. gen. size 0.77 ± 0.04) were also found in plantations (n = 1) and invaded sites (n = 2). In Baringo County, individuals that were morphologically identified as *P. juliflora* were mostly tetraploid (rel. gen. size 1.80 ± 0.02; n = 31). Occasionally, triploid individuals (rel. gen. size 1.36 ± 0.02) were detected in both plantations (n = 3) and invaded sites (n = 6). One diploid individual (rel. gen. size 0.95) was detected in one invaded site (Suppl. material 2). We found tetraploid *P. juliflora* individuals dominating invaded sites compared to triploid-diploid individuals (χ^2 = 23.22, df = 2, P < 0.001). All individuals identified morphologically as *P. pallida* were diploid (rel. gen. size 0.93 ± 0.03).

From the "overall" STRUCTURE analysis, *P. juliflora* individuals identified morphologically were largely assigned to the '*P. juliflora*' cluster (86.9% of 650 individuals, q_{ik} values ≥ 0.99; blue cluster in Fig. 3a). This cluster contained most of tetraploid (18 of 20 individuals) and triploid individuals (7 of 10 individuals) as well as all diploid individuals (n = 3). *Prosopis juliflora* individuals identified morphologically and showing some level of admixture (11.7% of 650 individuals; q_{ik} values ranging from 0.21 to 0.87 to the '*P. juliflora*' cluster) were mostly from plantations (n = 59), with comparatively few individuals from sites neighbouring plantations (n = 2) and invaded sites (n = 16) in Baringo County and only one individual was from the Afar Region. Hybrid individuals (0.12% of 650 individuals; i.e. showing equal proportions of genomic makeup from the two species; mean q_{ik} = 0.51 to the '*P. juliflora*' cluster) were only identified in Baringo County, from plantations (n = 4), sites neighbouring plantations (n = 1) and invaded sites (n = 3). A few triploid individuals (2 of 10 individuals) showed some level of admixture or were hybrids, while one triploid individual from Baringo County was assigned to the '*P. pallida*' cluster. Individuals identified morphologically as *P. pallida* were mainly assigned to the '*P. pallida*' cluster (95.1% of 61 individuals; q_{ik} values ≥ 0.99; orange cluster in Fig. 3a) or were founder hybrids (i.e. showing equal propor-

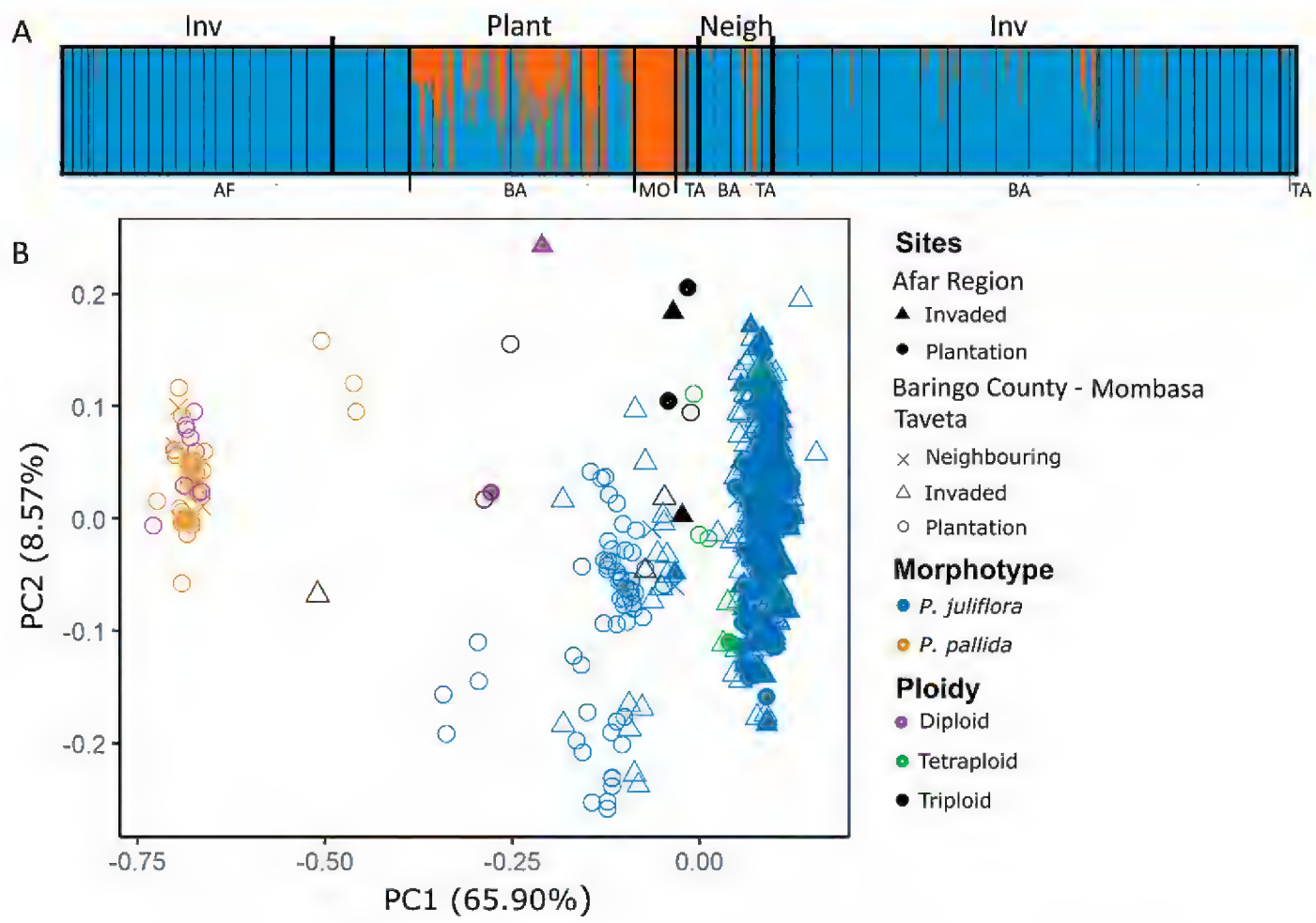

Figure 3. Genetic structure among *Prosopis* individuals from Kenya and Ethiopia based on STRUCTURE and Principal component analyses **A** STRUCTURE bar plots where vertical line plots illustrate the proportional assignment (q_{ik} values) of individual genomes to the inferred two genetic clusters, cluster 1 in blue and cluster 2 in orange; and for all *P. juliflora*, *P. pallida* morphotypes from plantations (Plant), sites of neighbouring plantations (Neigh) and far-off invaded sites (Inv) from the Afar Region in Ethiopia (AF), Baringo County (BA), Mombasa (MO) and Taveta (TA) in Kenya **B** principal component analysis (PCA) showing *P. juliflora* and *P. pallida* morphotypes and ploidy of individuals from flow cytometry results (see text for further details and Suppl. material 2). PCA was performed using Bruvo distances calculated using PolySat (Bruvo et al. 2004).

tions of genomic makeup from the two species; mean q_{ik} = 0.47 to the '*P. pallida*' cluster). All diploid individuals were assigned to the '*P. pallida*' cluster (n = 9). These STRUCTURE results were corroborated by the PCA analysis based on pairwise distances between individuals performed using Bruvo distances (Fig. 3b). The multivariate analysis revealed two main clusters along PC1, which captured 65.9% of the variation. One cluster consisted of *P. juliflora* morphotypes and tetraploids, while the other comprised *P. pallida* morphotypes and diploids. Some diploid and triploid individuals identified morphologically as *P. juliflora* were not distinctly assigned to either cluster. Species morphotype exhibited a strong association with PC1 (square correlation coefficient of 86.8%), while the correlation of invasion stages and countries with PC1 was minimal (20.1% and 0.04%, respectively).

The results of the "*P. juliflora*-only" STRUCTURE analysis in both countries identified two genetic clusters (hereafter referred to as "green" and "pink" clusters, Fig. 4a, Suppl. material 4) but they did not reveal any geographic patterns (i.e., country-specific clusters) or had associations with invasion stages (i.e., plantation vs invaded sites). Multivariate analyses, however, appeared to identify genetic subclustering (Fig. 4b), but these clusters were not related to either invasion stages (squared correlation coefficient of 9.9% with PC1 and 1.2% with PC2) or countries (squared correlation coefficient of 1.0% with PC1 and 1.6% with PC2). Interestingly, when evaluating the association between the genetic assignment of individuals via the STRUCTURE analysis, and their frequency in different invasion stages, most of the invasive genotypes in Baringo County were assigned to the

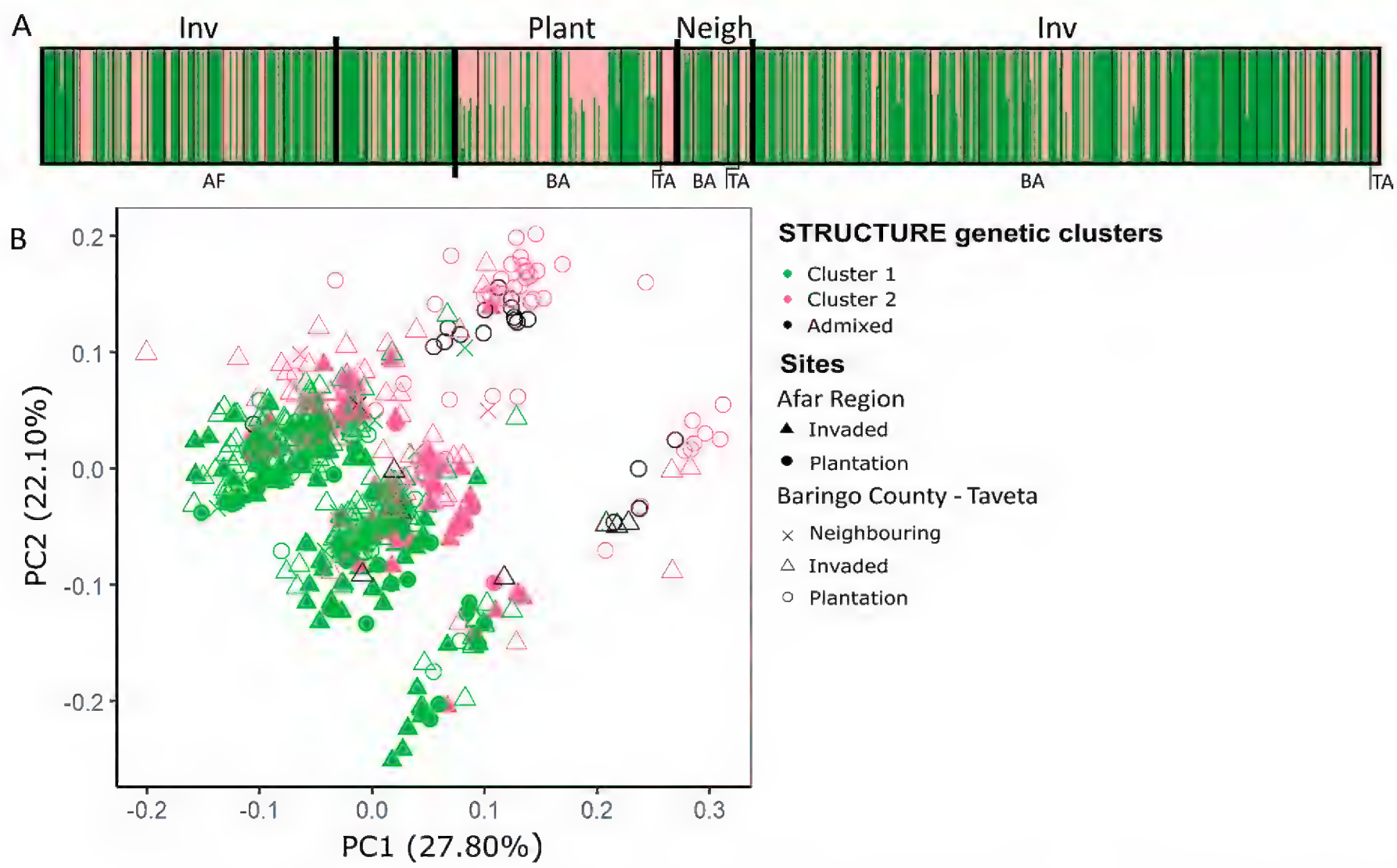

Figure 4. Genetic structure among *P. juliflora* individuals from Kenya and Ethiopia based on STRUCTURE and Principal component analyses **A** STRUCTURE bar plots for *P. juliflora* individuals from plantations (Plant), sites neighbouring plantations (Neigh) and far-off invaded (Inv) from the Afar Region in Ethiopia (AF), Baringo County (BA) and Taveta (TA) in Kenya (see text for further details). Vertical line plots represent the assignment (q_{ik} values) of individual genomes to the two inferred genetic clusters **B** principal component analysis (PCA) showing the assignment of individuals to the inferred STRUCTURE genetic clusters. PCA was performed using Bruvo distances calculated using PolySat (Bruvo et al. 2004).

"green" genetic cluster (for the Afar Region, 68.30% of 123 individuals assigned to "green" cluster, Fisher exact test P = 0.64 and for Baringo County, 81.40% of 258 individuals assigned to "green" cluster, Fisher exact test P < 0.001); in contrast, the assignment of founder individuals to both genetic clusters was similar (for the Afar Region, 31.70% of 123 individuals and 35.21% of 71 individuals assigned to "green" and "pink" clusters, respectively; and for Baringo County, 12.79% of 258 individuals and 34.56% of 136 individuals assigned to "green" and "pink" clusters, respectively). Almost all individuals from Taveta were assigned to the "pink" cluster and individuals not assigned to a single genetic cluster were all from Baringo County and mostly founder individuals (70.37% of 27 individuals).

Genetic insights into different invasion stages

Overall, low allelic richness was found in both species and in both countries (Table 1). In Baringo County, Kenya, values of A_R, H_O and H_E were higher in *P. juliflora* than *P. pallida* founder individuals in plantations. In addition, plantation individuals of *P. juliflora* had higher values of A_R, H_O and H_E than *P. juliflora* individuals from neighbouring and far-off invaded sites (Fig. 4a, b, c, respectively). The latter two also differed in levels of H_O (Fig. 5). In the Afar Region, *P. juliflora* individuals from plantations had similar A_R, H_E, H_O and F_{IS} to those from invaded sites. In comparison with plantation *P. juliflora* individuals in Baringo County, plantations in the Afar Region had lower values of A_R, H_O and H_E. Invasive *P. juli-*

flora individuals from Baringo County had similar values of A_R, H_E, H_O and F_{IS} to those from the Afar Region (Fig. 5).

No differences in genetic differentiation (pairwise F_{ST}) were found between different invasion stages in the Afar Region (P = 0.33; Fig. 6a) while for Baringo County, genetic differentiation between populations from invaded sites was 19% lower than between plantation and invasive populations, with substantial variability around their respective averages within each group (P < 0.05; Fig. 6b; i.e., the effect size is modest with Cohen's d = 0.44). For the Afar Region, AMOVA results revealed low genetic variation among invasion stages (8.42%) and among populations (12.92%), while most of the genetic variation was present within populations (78.66%, Table 2). For Baringo County, a similar amount of genetic variation was found among the invasion stages (10.32%) and among populations (13.70%), while most of the genetic variation resided within populations (75.98%, Table 2).

Table 1. Genetic diversity indices for *Prosopis* populations from Baringo County, Kenya and Afar Region, Ethiopia. Indices are given for plantations, areas neighbouring plantations and invaded sites far away from plantations (see text for further details). Statistics were calculated as mean values of each index over the seven microsatellite loci analysed. N = number of samples; A_R = Allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = inbreeding coefficient.

Sites	Species	Invasion stages	N	A_R	H_O	H_E	F_{IS}
Afar Region	*P. juliflora*	Plantation	64	1.62	0.41	0.34	0.05
	P. juliflora	Invaded	130	1.66	0.43	0.36	0.09
	P. juliflora	**All**	**194**	**2.36**	**0.42**	**0.35**	**0.08**
Baringo County	*P. juliflora*	Plantation	99	1.87	0.50	0.46	0.16
	P. juliflora	Neighbouring	22	1.67	0.41	0.36	0.17
	P. juliflora	Invaded	300	1.70	0.46	0.38	0.06
	P. juliflora	**All**	**421**	**2.96**	**0.47**	**0.41**	**0.12**
	P. pallida	Plantation	31	1.42	0.15	0.20	0.27
	P. pallida	Neighbouring	4	1.21	0.41	0.36	0.17
	P. pallida	**All**	**35**	**2.2**	**0.13**	**0.22**	**0.40**

Table 2. Hierarchical AMOVA of *P. juliflora* populations. Partitioning of genetic variation is given for different invasion stages (plantations, sites neighbouring plantations and invaded sites far away from plantations) in the Afar Region, Ethiopia and Baringo County, Kenya (see text for further details).

Source of variation	d.f.	Sum of squares	Variance	Percent variation (%)	Fixation index
Afar Region					
Among invasion stages	1	0.03	8.53	8.42	-0.02
Among populations	20	1.03	13.08	12.92	0.19*
Within populations	172	2.97	79.67	78.66	0.17
Baringo County					
Among invasion stages	2	1.21	12.36	10.32	0.19***
Among populations	30	1.58	16.39	13.70	0.10***
Within populations	388	8.90	90.92	75.98	0.27

Statistical significance: *, P < 0.05; ***, P < 0.001. Testing was done using 10,000 random permutations.

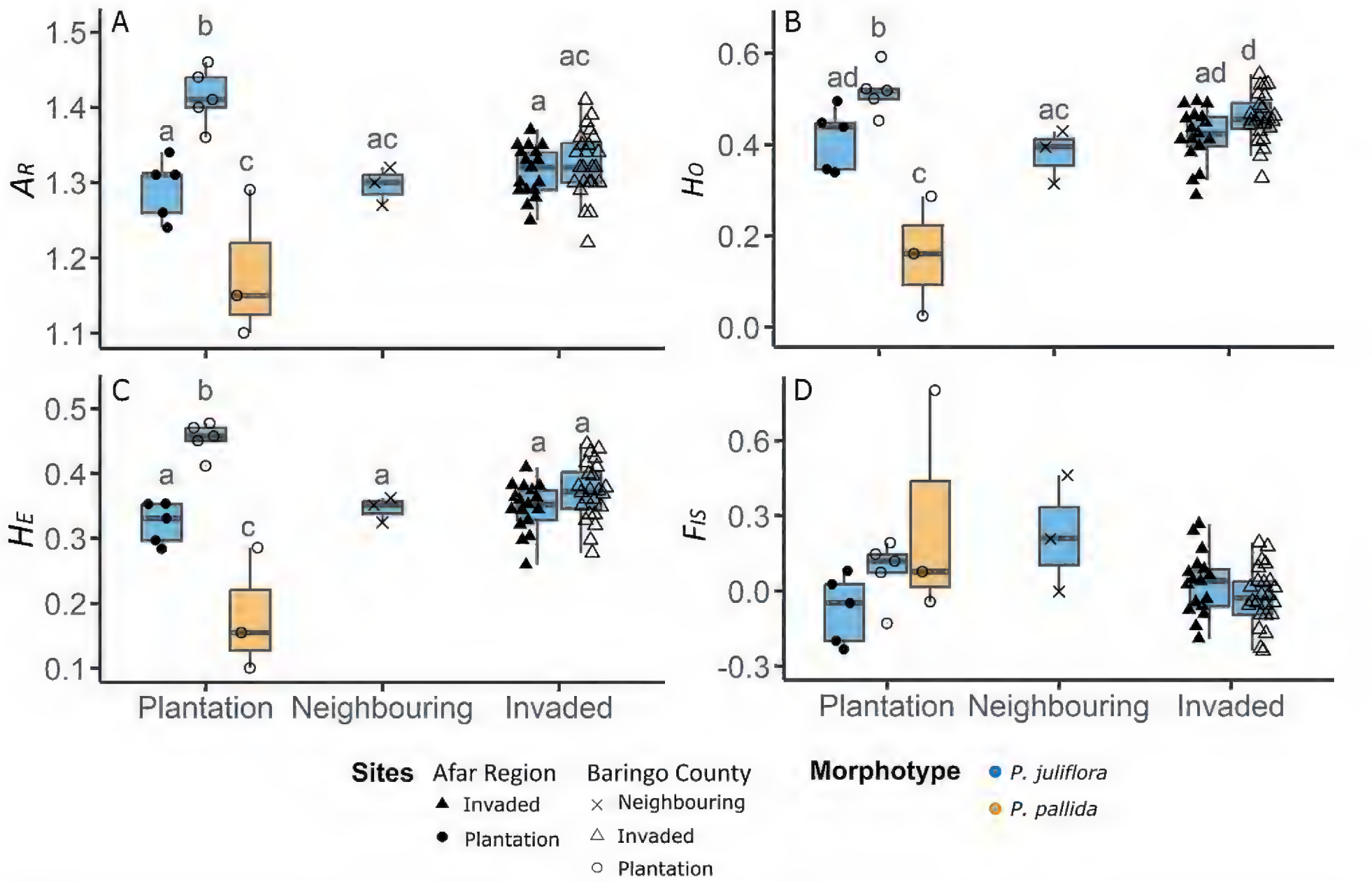

Figure 5. Genetic diversity metrics from different *Prosopis* invasion stages in the Afar Region, Ethiopia and Baringo County, Kenya. Genetic diversity metrics of *Prosopis juliflora* populations found in plantations, sites neighbouring plantations and far-off invaded sites in the Afar Region, Ethiopia and Baringo County, Kenya (see text for further details). Metrics for *P. pallida* plantations in Baringo County, Kenya are also shown **A** allelic richness (A_R) **B** Observed heterozygosity (H_O) **C** expected heterozygosity (H_E) **D** inbreeding coefficient (F_{IS}). Boxplots depict the median value, interquartile ranges, minimum and maximum of each region with population datapoint. Different letters indicate significant differences (nonparametric bootstrap t-test; $P < 0.05$) between the corresponding groups.

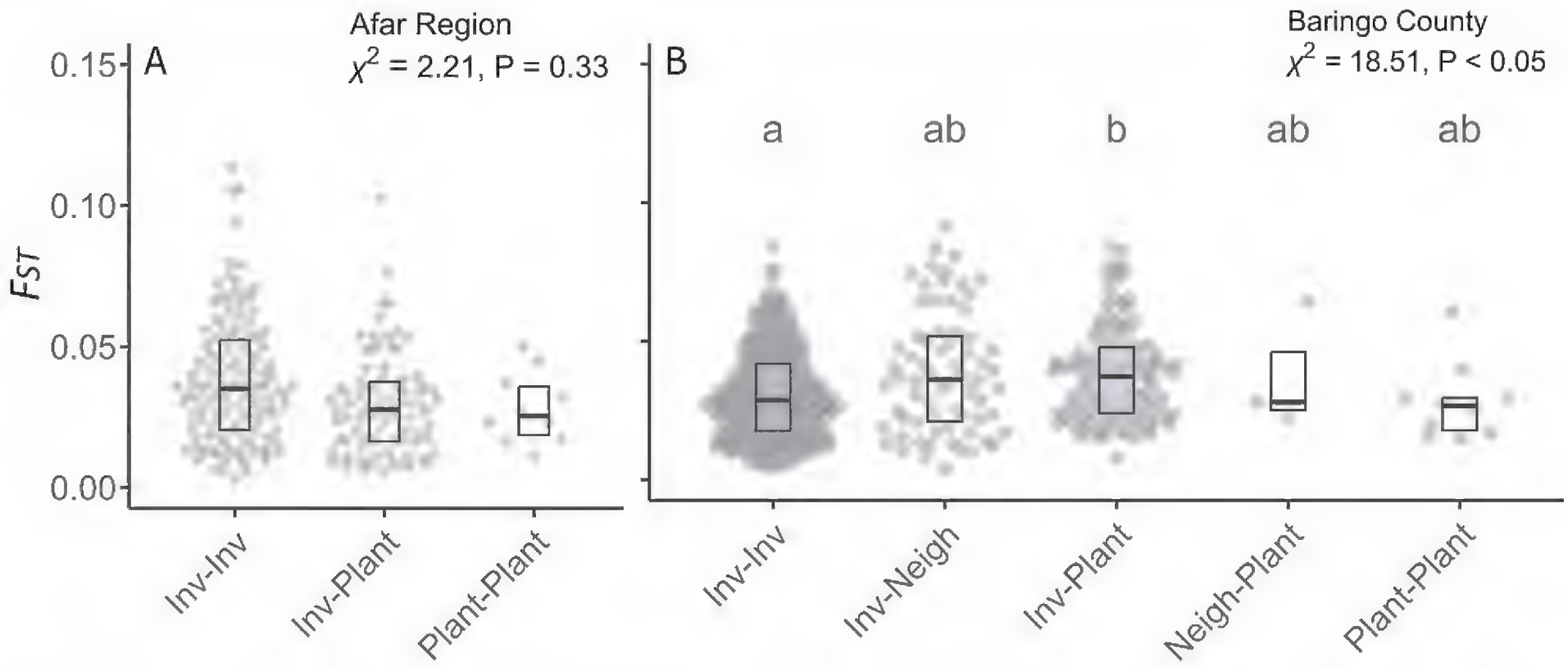

Figure 6. Comparison of F_{ST}-based genetic differentiation between *Prosopis juliflora* populations in **A** the Afar Region, Ethiopia and **B** Baringo County, Kenya. Boxplots and data points show the distribution of pairwise F_{ST} between pairs of populations from invaded sites (Inv-Inv), invaded and sites neighbouring plantations (Inv-Neigh), invaded and plantation sites (Inv-Plant), plantations and sites neighbouring plantations (Neigh-Plant), and plantations sites (Plant-Plant) (see text for further details). The inserted boxplot depicts the median value, and interquartile ranges. Different letters indicate significant differences (Kruskal-Wallis rank sum test; $P < 0.05$; Bonferroni-Dunn post hoc test).

Effect of environmental variables on dispersal at invasive population level

Pairwise F_{ST} values between populations (excluding plantations) were not related to the geographical distance in Baringo County or in the Afar Region (Table 3). In both countries, the environmental resistance modelling approach indicated no significant relationships between pairwise F_{ST} values and any of the pairwise ecological distances based on the environmental variables considered (Table 3). Thus, dispersal between invasive populations in both study areas was not affected by any of the environmental variables tested.

Discussion

A good understanding of the eco-evolutionary factors and dynamics that underlie the invasion success of alien species can help designing effective control methods. We evaluated genetic and ecological aspects that may promote or impede invasion by *Prosopis* trees in eastern Africa by using a rare opportunity to examine and compare the genetic characteristics of founding populations (i.e., ancestral trees) and their invasive populations (i.e., descendant trees). We found support for our hypothesis that different stages of invasion are dominated by different genetic characteristics due to site-specific demographic dynamics, such as those affecting genetic admixture during rapid range expansion. In Baringo County, but not in the Afar Region, this was expressed by different stages of *P. juliflora* invasion being dominated by different genotypes. We did not find support for the hypothesis that dispersal between invasive populations is affected by environmental variables in both study areas, at least at the spatial scales included in our study.

Genetic insights across different invasion stages

As expected, different site-specific demographic dynamics characterise the invasion in Afar Region and Baringo County. In the Afar Region, no differences in genetic

Table 3. Results of Mantel tests and Partial Mantel tests to assess correlations between various landscape variables, geographic distances and pairwise F_{ST} between *P. juliflora* populations. Tests were performed among all *P. juliflora* populations from the Afar Region, Ethiopia and Baringo County, Kenya. Test statistics (r values) and significance levels (P values) are provided.

Tested relation	Afar Region		Baringo County	
	Mantel test		Mantel test	
	r	P	r	P
F_{ST} x geographic distance	0.01	0.83	0.01	0.51
F_{ST} x elevation†	0.05	0.15	0.01	0.70
F_{ST} x Annual precipitation†	0.05	0.13	0.01	0.81
F_{ST} x Precipitation of wettest quarter†	-	-	0.01	0.68
F_{ST} x Mean temperature of driest quarter†	-	-	0.01	0.63
F_{ST} x Max temperature of warmest month†	-	-	0.01	0.63
F_{ST} x Distance to rivers†	0.01	0.86	0.09	0.06
F_{ST} x Distance to roads†	0.01	0.78	0.02	0.45
F_{ST} x Distance to villages†	0.03	0.31	0.01	0.57
F_{ST} x Distance to livestock markets†	-	-	0.02	0.55

† Partial mantel test with geographic distance as covariable.

diversity were found between invasive and founder trees. In contrast, in Baringo County, invasive *P. juliflora* individuals and individuals near founding populations were genetically less diverse than founder trees. Our findings agree with a previous study showing similar levels of genetic diversity in native populations of *P. juliflora* in Mexico and Ethiopian populations, while Kenyan populations had higher genetic diversity than Mexican ones (Castillo et al. 2021a). Taken together, this suggests that multiple introduction events, or a single introduction from a highly admixed population (Le Roux et al. 2010), founded these populations. Our analyses did not find *P. juliflora* to be genetically structured according to the invasion stage. However, in Baringo County, the assignment of individuals to one of the two identified genetic clusters correlated with being invasive and the genetic variation observed at different invasion stages was similar to that at population level. These results are in line with the hypothesis that different invasion stages (i.e., introduction, naturalisation and spread) are dominated by different genotypes. In theory, it is likely that invasive populations of any species include genotypes of differential fitness (Richardson and Pyšek 2006; Theoharides and Dukes 2007; Zenni et al. 2014). An alternative explanation is that colonisation of new areas away from plantations involved only a few individuals which could erode the genetic diversity along the route of expansion due to consecutive founding events (Austerlitz et al. 1997; Dlugosch and Parker 2008). The higher genetic diversity in founder individuals in Baringo County may provide higher adaptive potential during invasion compared to the lower genetic diversity of founder individuals in the Afar Region. Indeed, Castillo et al. (2021b) showed that in Baringo County rapid evolution in *P. juliflora* likely increased the species' invasiveness, which provides further support for the hypothesis that different genotypes may underlie different invasion stages. This might also indicate that the selective forces operating during the invasion process of *Prosopis* in Baringo County are absent in the Afar Region.

Genetic diversity is affected by patterns of gene flow (i.e., genetic admixture) among populations (Lachmuth et al. 2010; Sexton et al. 2011; Li et al. 2016; but see Matesanz et al. 2014). In Baringo County, F_{ST}-based genetic differentiation was higher between founder and invasive populations than between populations in invaded sites, while no difference in genetic differentiation was evident for populations in the Afar Region. Often, small outlying colonising populations may rely on gene flow from "source" populations for their successful establishment and further spread, in what has been termed "genetic rescue" (Ingvarsson 2001). This is not the case for *Prosopis* invasions in eastern Africa. In small founder populations, gene flow may increase genetic diversity for selection to act upon (e.g., Sexton et al. 2011). In our case, ongoing gene flow between founding *P. juliflora* individuals in the Afar Region and the invasive individuals may have led to the homogenisation of the standing genetic diversity.

The effects of environmental variables on dispersal at invasive population level

Effective dispersal leads to gene flow and therefore, the structuring of genetic variation observable through the spatio-temporal distribution of alleles (Broquet and Petit 2009). For instance, when dispersal is primarily diffusive, a pattern of isolation-by-distance (Wright 1943), whereby populations are genetically more differentiated at larger geographical distances than at smaller distances, may result. Alter-

natively, dispersal can be higher between populations under similar environmental conditions, termed isolation-by-environment (Wang and Bradburd 2014). Numerous species, including invasive plants, display patterns of isolation by distance or by environment, or both, across environmental gradients (Sexton et al. 2014; Cornille et al. 2016; Gallego-Tévar et al. 2019; but see VanWallendael et al. 2021). In our study, genetic differentiation between invasive populations of *P. juliflora* in the Afar Region and Baringo County was low and not correlated with geographic distance. Even if the landscape and climatic variables tested here determine the current potential suitable habitats of *P. juliflora* in eastern Africa (Shiferaw et al. 2019; Eckert et al. 2020; Mbaabu 2023) and linear landscape variables such as rivers and roads are important to predict the future spread of *Prosopis* (Shiferaw et al. 2019); they have no impact on dispersal over small spatial scales, or at early invasion stages as it has been proposed for the Afar Region (Shiferaw et al. 2019).

To disentangle the roles of rapid range expansion and the environmental factors that affect dispersal in determining population genetic structure is difficult (Cushman 2015). The spread of *Prosopis* in the Afar Region seems to involve local expansion from plantations as well as human-assisted long-distance dispersal events (Shiferaw et al. 2019). Since our sample strategy likely did not include populations at the invasion front, our results probably reflect the genetic structure that resulted from local spread from plantations (although secondary contact could also have occurred). Introduced *Prosopis* species are frequently dispersed by livestock often over substantial distances (Pasiecznik et al. 2001). Such long-distance dispersal events, commonly linked to animal and human vectors and linear landscape variables (Petit et al. 2004), may also explain the lack of isolation-by-resistance in our study areas. Another factor to consider is the relatively small spatial scale of both study areas (Baringo County covers an area of 1,015 km^2 while Afar Region covers an area of 270,000 km^2; Bekele et al. 2018). The invaded areas we included in our study may therefore be smaller than the spatial scales over which dispersal typically operates in *Prosopis* (Pasiecznik et al. 2001). Future studies assessing the genetic dynamics of *Prosopis* invasion both within and beyond the margins of the spread from plantations (i.e., by involving genotypes that did not originate from planted trees) would enhance the understanding of the eco-evolutionary dynamics of its invasion success.

Genetic comparisons between *P. juliflora* and *P. pallida*

We found higher allelic richness in founder individuals of *P. juliflora* than in *P. pallida* founders. We also found evidence for hybridisation between the two species in Kenya, but many of these hybrids had not spread beyond the original plantations. This indicates that hybrid individuals possibly resulted during the cultivation of these two species in Kenya or elsewhere and were planted at our study sites. The two species occur allopatrically over most of their native ranges (Pasiecznik et al. 2001; Palacios et al. 2012; Ramírez 2015).

While the success of many invasive alien plants has been attributed to hybridisation (Schierenbeck and Ellstrand 2009; Zalapa et al. 2010; Gaskin et al. 2012), this has clearly not been the case for *Prosopis* in eastern Africa. The low number of invasive hybrids we detected should not be surprising given the difference in ploidy levels between *P. juliflora* and *P. pallida*. Closely-related species with different ploidy levels can often co-exist since reproductive isolation is maintained through hybrid sterility (Petit et al. 2004). In some cases, fertility can be restored

through chromosome doubling of triploid hybrids (Campos et al. 2009) and apomixis (Verduijn et al. 2004; Mráz and Zdvořák 2019). In the case of *P. juliflora* and *P. pallida* some hybrids were, as expected, triploid, and presumed to be sterile. In contrast, *Prosopis* invasions in Australia and South Africa are dominated by hybrid swarms that originated from interbreeding between various diploid *Prosopis* species (van Klinken et al. 2006; Mazibuko 2012; *P. juliflora* is the only polyploid species in the genus). We also found some triploid and diploid individuals from plantations and invaded sites to be assigned to the "*P. juliflora*" cluster. One possible explanation for this is that genome reduction might be occurring in *P. juliflora* as has been documented for other polyploid plants (Lavergne et al. 2010; Wendel 2015).

Polyploidy is an important evolutionary force in flowering plants, with one-third of all angiosperms being descendants of polyploids (Wright 1962). Polyploidy has also been repeatedly linked to plant invasiveness (Pandit et al. 2011; te Beest et al. 2012), where species with higher ploidy levels are more likely to invade new areas than diploid ones (Nagy et al. 2017). This is due to variation in genome size leading to changes in phenotypic responses (Beaulieu et al. 2007; Lavergne et al. 2010; Williams and Oliveira 2020), adaptive variation (te Beest et al. 2012) and colonisation potential (Lavergne et al. 2010; te Beest et al. 2012). Overall, these benefits of polyploidy may explain the successful invasion of *P. juliflora*, and not *P. pallida*, in Baringo, Kenya.

Implications for management

We found that dispersal by invasive *Prosopis* trees in eastern Africa is not impeded in any way by climatic conditions, linear landscape variables or variables related to anthropic features. In fact, linear landscape variables seem to be promoting *Prosopis* invasion. It can thus be expected that all available habitats, defined by environmental variables we tested (or similar to those at sites that have already been invaded), will be equally susceptible to invasion, unless the vectors of spread (humans and their livestock, and wildlife) can be controlled. Long-distance dispersal should be considered when designing management options for *Prosopis* invasions. Management efforts should focus on reducing seed production, dispersal into areas where *Prosopis* is currently not found, and the movement of propagules along linear landscape/anthropic variables. It is probable that attempts to slow *Prosopis* spread and deal with dense-invaded areas through utilisation in some areas such as Baringo County (Choge et al. 2002) have been ineffective (Mbaabu et al. 2019) because they do not prevent the production and dispersal of *Prosopis* seeds. In fact, they may even promote the spread of seeds, because people would be tempted to plant in areas where it does not yet occur in order to benefit from utilisation. Because of this, effective control of these invasions would have to include a combination of integrated methods such as biological, chemical and mechanical control.

The use of biological control agents has been proposed as a safe, sustainable, cheap and effective way to reduce the spread of *Prosopis* (van Wilgen et al. 2012; Shackleton et al. 2014). Co-evolved biological control agents that are pre-adapted to a particular species may be less effective against hybrids (Goolsby et al. 2006; Smith et al. 2018; Jourdan et al. 2019; Paterson et al. 2019). In eastern Africa, we found that invasive *Prosopis* individuals are not hybrids, which could increase the chances of finding effective biological control agents that are specific to *P. juliflora*. In South Africa, research into the biological control of *Prosopis* is now focused on a suite of new poten-

tial agents (Kleinjan et al. 2021). Kleinjan et al. (2021) note that "… potential agents that damage the vegetative growth of the plants have been included in response to recognition in South Africa, that there is no other route to successful management of *Prosopis*". We strongly recommend that a similar approach be adopted in eastern Africa. Our study adds evidence to previous research showing that polyploidy may have been linked to the invasion success of *P. juliflora* in eastern Africa. Therefore, unintentional and intentional trans-border movement of these species should be prohibited, especially since ongoing climatic change is expected to increase the risk of invasion by the species into neighbouring countries (Eckert et al. 2020).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

The dataset analysed during the current study is available in https://doi.org/10.5281/zenodo.10951565.

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Supplementary material 1

Sampling sites of *Prosopis* individuals from Kenya and Ethiopia included in the study

Authors: María L. Castillo, Urs Schaffner, Purity R. Mbaabu, Hailu Shiferaw, Brian W. van Wilgen, Sandra Eckert, Simon Choge, Zuzana Münzbergová, Johannes J. Le Roux

Data type: docx

Explanation note: Sampling sites of *Prosopis* individuals from Kenya and Ethiopia included in the study. For each site, the following is indicated: locality, sample site ID; site category, i.e., plantation, sites neighbouring plantations, and invaded sites far away from plantations; the *Prosopis* species found in each sample site; the number of individuals of each species sampled (N); and geographic coordinates in decimal degrees. Sites neighbouring plantations have the same ID and coordinates as their corresponding plantation sites. Sites neighbouring plantations include trees located at a distance of less than 100 m from founder-planted trees.

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Supplementary material 2

Flow cytometry results for *Prosopis* individuals from the Afar Region, Ethiopia and Baringo County, Kenya

Authors: María L. Castillo, Urs Schaffner, Purity R. Mbaabu, Hailu Shiferaw, Brian W. van Wilgen, Sandra Eckert, Simon Choge, Zuzana Münzbergová, Johannes J. Le Roux

Data type: docx

Explanation note: Flow cytometry results for *Prosopis* individuals from the Afar Region, Ethiopia and Baringo County, Kenya. For each individual is indicated the ID sample site; species morphological identification, site category, i.e., plantation, sites neighbouring plantations, and invaded sites far away from plantations; expected cytotype and relative genome size values.

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Link: https://doi.org/10.3897/neobiota.93.117457.suppl2

Supplementary material 3

Information of environmental variables used to evaluate their effect on the dispersal of invasive populations of *P. juliflora* in the Ethiopian Afar Region and Baringo County, Kenya

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Data type: docx

Explanation note: Description, processing and/or sources of environmental variables used to evaluate their effect on the dispersal of invasive populations of *P. juliflora* in the Ethiopian Afar Region and Baringo County, Kenya.

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Supplementary material 4

Identification of the optimal number of clusters (K) for *P. juliflora* individuals

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Data type: docx

Explanation note: Identification of the optimal number of clusters (K) for *P. juliflora* individuals. Analysis was done on the invaded areas of the Afar Region in Ethiopia, Baringo County and Taveta in Kenya, inferred by Bayesian clustering with the software STRUCTURE Harvester (Earl and vonHoldt 2012) and CLUMPAK software (Kopelman et al. 2015) was used to graphically display of the results. Data set contains a total of 630 individuals for seven nuclear microsatellites loci (see Material and Methods for parameters of the models).

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